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Mini-review

HnRNP PARTICLES

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Introduction

In the last several years a variety of new observations have appeared which bear on the *in vivo* complexing of eukaryotic messenger RNA (mRNA) sequences with proteins in the nucleus and cytoplasm. Our purpose in this article is to review only the recent experimental evidence relevant to the problem of the proteins complexed with heterogeneous nuclear RNA (hnRNA).

This essay is divided into four sections. Part 1 describes the methods currently used to isolate hnRNP particles and the problems associated with it. Part 2 deals with the RNA portion of the hnRNP particles, while part 3 is devoted to the proteins found to be associated with hnRNA. In the final section we review current knowledge regarding the function and structure of these particles.

1. Isolation of hnRNP particles

To obtain pure hnRNP particles it is first necessary to start with the isolation of a nuclei preparation which is not contaminated with cytoplasmic material. This criterion can be met, for example, by centrifugation of the cell lysate through heavy sucrose solutions (1) which may be followed by successive washings of the nuclei with buffer solutions containing low concentrations of non-ionic detergents (2, 3, 4).

Two procedures for the isolation of crude hnRNP particles are now widely used. In the first procedure, originally described by Samarina et al. (5), rat liver nuclei were first extracted with a neutral buffer and subsequently extracted several times with the same buffer at pH 8.0 in the presence of a ribonuclease inhibitor. The combined pH:8.0 extracts contained hnRNP particles with sedimentation coefficients in sucrose gradients ranging from 30 - 200 S and CsCl densities of 1.39 - 1.40 g/cm³. The presence of the

ribonuclease inhibitor during the extraction was essential since only particles of about 30 S were found in the absence of the inhibitor. This extraction method has been used and modified by several workers (2, 6-14). An interesting modification was found by Ishikawa et al. (7) who were able to extract hnRNP particles from rat liver nuclei at neutral pH by adding ATP to the extraction buffer. Since EDTA and pyrophosphate could be substituted for ATP, it seems that it is the chelating capacity of the ATP that is involved in the release of hnRNP from chromatin (3).

A second method for the isolation of hnRNP particles was described by Parsons and McCarty (15, 16). Washed rat liver nuclei were sonicated in a buffered 0.35 M sucrose solution (16) and after removal of membranes and nucleoli the hnRNP particles were purified via sucrose gradient centrifugation. The hnRNP particles present in the nuclear lysate obtained either by sonication or extraction with buffer are usually purified by sucrose gradient centrifugation although in some cases affinity chromatography has been used (8, 17-19).

The sonication method has been used in the isolation of hnRNP particles from a wide variety of normal and transformed cells (3, 8, 9, 13, 18, 20-25), in contrast to the extraction method which seems less widely applicable. For KB cells and HeLa cells, for instance, a higher temperature was needed (2, 8, 10) and for ascites tumor cells a higher pH was more favourable (11). It was further observed that with some fast growing cell types (e.g. rat hepatoma cells) only the sonication method yielded significant amounts of hnRNP particles (8, 21, 23). Other advantages of the sonication method over the extraction method are its rapidity and the fact that it can be used at low temperatures thus diminishing the possibility of degradation of hnRNP.

The hnRNP particles (40-250 S) found after

sonication had a similar CsCl density as the particle population isolated by the Samarina method (5). Degradation of these intact hnRNP structures by endogenous or added ribonuclease released monomer RNP particles of about 30 S. These 30 S subparticles (30 - 40 S according to some authors (2, 24)) have an RNA to protein ratio of about 1:4 to 1:5 as indicated by their buoyant density of 1.40 g/cm³ in CsCl (5, 8, 26, 27). Samarina and coworkers (5, 26, 28) proposed that these 30 S subparticles were composed of a residual stretch of hnRNA attached to a globular protein particle, called an informoer (5, 26). An informoer was thought to be a complex of a number of (identical) proteins with a subunit molecular weight of about 40,000 (26, 29).

The 30 S RNP subparticles show a rather homogeneous peak in sucrose gradients (2, 5, 26) and electronmicroscopic observations revealed them to be rather homogeneous particles of about 200 - 300 Å in diameter (26, 30-32). Treatment of brain nuclear hnRNP particles with increasing concentrations of ribonuclease, however, suggested the existence of different classes of monomer particles (33) with different protein composition (24). Several reports demonstrate that some of the 30 S RNP proteins are more tightly (mostly interpreted as more specifically) bound than others (2, 24), a characteristic that can easily lead to a heterogeneous population of 30 S subparticles. It would be worthwhile trying other techniques (for example metrizamide gradients (9, 25), high resolution CsCl gradients (34, 35) or polyacrylamide-agarose composite gel electrophoresis (36)) in order to demonstrate the microheterogeneity of these particles.

2. The RNA in hnRNA and hnRNP

This chapter will be limited to summarizing the known facts about the characteristics of hnRNA and its relation to cytoplasmic mRNA. Several excellent reviews are available which consider the evidence underlying these conclusions (37-40).

a. The presence of mRNA sequences

The rapidly synthesized hnRNA is found complexed with proteins (5, 6, 8, 41), and the hnRNP particles isolated as described in the previous section contain the bulk of the hnRNA molecules (26, 28, 42). Hybridization experiments between RNA or DNA complementary to mRNA and purified hnRNA (15, 38, 40, 43-47) indicate that mRNA sequences are found in hnRNA molecules. Further evidence

on this point can be found in the successful translation of hnRNA in oocytes and cell-free systems (3, 48-50), from kinetic studies with and without transcriptional inhibitors (8, 38) and from the similarity in the types of posttranscriptional modification characteristic of hnRNA and mRNA (38). Although all these methods have their specific draw-backs (especially concerning the purity of the mRNA and hnRNA preparations used) all these experiments taken together provide strong evidence for the occurrence of mRNA sequences in hnRNA. In fact, recent work with carefully purified RNA preparations from globin synthesizing cells (mouse spleen, fetal mouse liver and mouse Friend cells) led to the identification of the immediate precursor of 9 S globin mRNA as a 15 S component (51, 52). Furthermore, Bastos and Aviv have shown that this 15 S component is a nuclear cleavage product of a still larger precursor of about 27 S (53).

Recent findings also suggest that probably all poly(A)-containing mRNA sequences found in the cytoplasm are present in hnRNP-RNA (and notably in the informoer-like 30 S RNP particles) while on the other hand only a small part of the hnRNP-RNA sequences can be detected in the cytoplasm (42, 44). This means that the greater part of the hnRNA molecules turns over within the nucleus, as has been suggested earlier by several workers on basis of kinetic data (54-57).

b. The presence of poly(A) and oligo(A) sequences

The 3'poly(A) segments characteristic of most cytoplasmic mRNAs have been shown to be present in hnRNA and hnRNP complexes (11, 17, 18, 39, 43, 58). The poly(A) is added posttranscriptionally by a terminal addition enzyme (see for example 59). It has been found by several authors that the poly(A), like the rest of hnRNA, is (partly) covered with protein and is released as a 10 - 16 S poly(A)-protein complex after ribonuclease degradation of hnRNP complexes (11, 58, 60, 61).

In addition to the long posttranscriptionally added poly(A) segments (150-200 nucleotides long) there are also short transcribed AMP-rich sequences present in hnRNA and hnRNP (37, 62, 63). These oligo(A) sequences (about 30 nucleotides long) seem to be primarily present in those hnRNA molecules that do not contain the long poly(A) sequences (37, 62). A considerable fraction of these oligo(A) stretches was found in 30 S hnRNP particles (42, 63). It was proposed that they might function as primers for polyadenylation (37, 63).

c. The presence of oligo(U) sequences

The presence of short oligo(U) sequences of about 30 nucleotides in hnRNA was first demonstrated by Molloy and coworkers (64) and shown not to be localized near the 3' terminus (65) although this point is still in dispute (22). There are indications that these sequences are present only in polyadenylated hnRNA and as such may contribute to the secondary structure of hnRNA (66).

Recently, oligo(U) sequences have been shown to be present in hnRNP complexes isolated either by sonication or by extraction at pH 9.0. In both cases (22, 61) the oligo(U) sequences were found in a ribonuclease resistant duplex with the poly(A) of the hnRNP particles after phenol-chloroform extraction of the hnRNA but there is some disagreement as to whether the oligo(U) sequences could become associated artefactually with the poly(A) of hnRNP during isolation. Quinlan and coworkers (61) showed that hnRNP-poly(A) from Taper hepatoma ascites cells could bind added poly(U) during RNA extraction and, conversely, that added poly(A) increased the yield of ribonuclease resistant oligo(U)-poly(A) duplexes. Although these results do not exclude the presence of oligo(U)-poly(A) duplexes in hnRNP, they do show that artefactual formation of such duplexes during preparative procedures is possible. Kish and Pederson (22) on the other hand concluded from their experiments with HeLa cells that the oligo(U)-poly(A) duplexes were genuine. They isolated these duplexes in the presence of an excess of oligo(dT) which was assumed (but not proven) to block the free poly(A) sequences of hnRNP completely from artefactual association with oligo(U) sequences present in the nuclear lysate.

d. The presence of (sn)RNA species

Recent reports from Sekeris and coworkers (12, 13, 67) indicate that in addition to the rapidly synthesized hnRNA molecules smaller, metabolically more stable, nuclear RNA species are present in undegraded rat liver hnRNP particles. These RNA molecules could be identical with some of the stable nuclear (sn)RNA species isolated by various authors from several types of cells (68, 69) and which have been shown to be present in RNP structures (70).

3. The proteins in hnRNP particles

There is an abundance of material concerning the protein composition of hnRNP complexes (2, 4, 8, 9,

13, 14, 17-21, 24, 25, 71-74). Since large discrepancies are found in the reports on the number and molecular weight distribution of the proteins associated with the hnRNA, however, it seems useful to first discuss some of the problems involved in this matter.

a. Cytoplasmic contamination (see section 2) should be kept as remote as possible. Not all workers purify the nuclei in the same way or to the same extent and this can easily introduce discrepancies in the protein patterns found.

b. Differences in the protein composition of hnRNP particles may be the direct result of differences in the isolation procedures. For example, the protein composition of 30S hnRNP particles in most cases is quite simple in contrast to that of intact hnRNP (see below). Uncontrolled endogenous ribonuclease activity during the isolation procedure may convert (specifically or non-specifically) hnRNP particles into 30S RNP particles and introduce unknown losses of hnRNP protein. Also, the uncontrolled presence of nuclear proteases can be a major problem in these experiments. Furthermore, it should be realized that most results were obtained with RNP particles purified by sucrose gradient centrifugation only, under conditions that do not effectively remove adsorbed proteins. For example, high salt washes are often omitted because hnRNP particles tend to disaggregate much easier in high salt solutions than cytoplasmic mRNP particles (2, 4, 8, 17, 71, 75). On the other hand, this property can be used advantageously as a measure of the specificity of the protein-hnRNA interaction as has been shown by Beyer et al. (2).

c. Some of the proteins associated with hnRNA may be species-specific. This possibility has been substantiated by the results obtained by Pederson (8, 17) and recently Beyer et al. (2). Other workers have found small quantitative changes in the protein composition of hnRNP particles which are thought to be induced by carcinogenic reagents (21, 76).

The protein composition of intact hnRNP particles is very complex as can be concluded from the analysis of hnRNP particles isolated by sonication (4, 9, 21, 24, 25), extraction or lysis in the presence of ribonuclease inhibitor (14, 27, 71, 73) or affinity chromatography (8, 17-19). Most workers find a series of proteins ranging in molecular weight from 22,000 to 150,000 with some predominant components around 40,000 (4, 8, 14, 19-21, 24, 73). Similar results were obtained when the hnRNP particles were subjected to a 0.5 M KCl wash (4, 8, 19, 71), a treatment that is

known to remove less tightly bound proteins from RNP particles.

The dominating protein bands of about 40,000 daltons present in undegraded hnRNP complexes are most probably identical with the 40,000 dalton proteins that are the structural components of the informofer-like 30S particles (23, 29). The use of high resolution polyacrylamide gel systems has shown that purified, high-salt-washed 30S particles from a variety of cultured cells contain at least two proteins having almost identical molecular weights around 40,000 daltons (2, 11, 60).

Recent reports have characterized some of these proteins. The major proteins of the 30S particles in HeLa cells (77), mouse ascites cells (60) and rat brain (24, 71) are subject to posttranslational phosphorylation and it has been shown that these phosphorylated proteins are more tightly bound to hnRNA sequences than the non-phosphorylated ones (71).

Some amino acid compositions of structural proteins in 30S subparticles have been published (2, 29). In rat liver the principal component of informofers is a more or less neutral protein (29), while the most abundant structural proteins in 30S subparticles from HeLa cells are basic polypeptides with a high glycine content and a modified arginine residue (2). Such an arginine residue with two methylgroups added to the guanidino group (N^G , N^G , dimethylarginine) has also been found in rat liver nuclear RNP (78). This is an interesting finding because the guanidino group of arginine may be involved in the interaction between proteins and nucleic acids (79, 80).

The interesting question whether some hnRNP proteins are identical with proteins present in cytoplasmic mRNP is still unresolved. Lukanidin et al. (81) used an immunological approach and concluded from their experiments that the 40,000 dalton protein present in rat liver informofers was absent from polysomal mRNP. Most workers, comparing the electrophoretic mobilities of hnRNP proteins and polysomal mRNP proteins, agree that the predominant proteins associated with polysomal mRNA are only minor proteins, if present at all, in the nuclear particles (4, 8, 19, 82, 83).

This also holds for the poly(A) associated 76,000 dalton protein which is one of the major proteins of polysomal mRNPs (84-86) but appears as only a very minor component of the HeLa cell hnRNP particles after oligo(dT)-cellulose purification and which in fact is not readily observed at all in electropherograms of total hnRNP protein (18, 19). It was found, however, as a major component after isolation of the

nuclear poly(A)-protein complexes from HeLa cells (18, 19), and in another system (the cellular slime mold *Dictyostelium discoideum*) a 73,000 dalton component was found to be associated with the poly(A) of hnRNP particles (17). From mouse ascites nuclei (Taper liver tumor) two classes of poly(A)-protein complexes were isolated (11, 60). One fraction, sedimenting at 17S, contained at least six proteins with molecular weights between 17,000 and 30,000 daltons. The second fraction (15S) had a predominant protein with a molecular weight of 80,000 daltons. Unfortunately, the possibility of cytoplasmic contamination was not rigorously excluded in either case. This is important, since the use of affinity chromatography (17-19) may (preferentially) co-purify cytoplasmic poly(A) containing mRNP.

4. Structure and function of hnRNP

A simple model for the structure of hnRNP complexes was the informofer model proposed by Samarina et al. (5, 28, 58). In this model the hnRNP particles were visualized as a number of globular particles composed of one main protein (40,000 daltons) attached to a heterogeneous population of RNA strands which had poly(A)-segments complexed with proteins on their ends (58). Although proteins certainly play an important role in the maintenance of hnRNP structure, more recent work points to a model in which the hnRNA is an integral part of the globular 30S particles described by Samarina. On the basis of sequential release of proteins from hnRNP particles by salt or ribonuclease treatments, a so called "folded ribonucleoprotein strand" structure was proposed by Stevenin and Jacob (24, 75, 87) and supported by others (12, 42). Several groups have shown that complex-formation of protein with hnRNA occurs during transcription (25, 41, 88-90) and electronmicroscopic observations have shown that long RNA molecules interact with protein during transcription to form repeating 200-240 Å particles with apparently equal spacing and size (31, 91). As a result of this association with protein the RNA is foreshortened considerably (30) and it seems that in this way the cell can handle (by condensing) and protect (by covering it with protein) long, unstable lengths of RNA while still leaving them available for further enzymatic modification. An identical situation appears in nucleosomes, where the DNA is considerably condensed but obviously still accessible, for instance to RNA polymerase (92). Additional

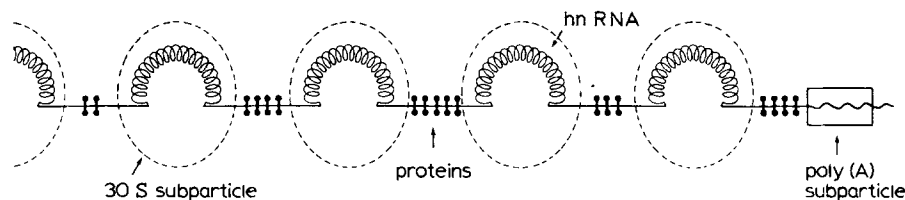


Fig. 1. Schematic drawing of a possible model of hnRNP structure.

stabilizing of the hnRNP network may be obtained by binding to nuclear structures as was suggested by Faiferman and Pogo (93).

A possible model of hnRNP structure is depicted in figure 1. It shows the 30S subparticles containing the majority of the hnRNA sequences (8, 28, 60) in a condensed form bound to a simple set of polypeptides. The ribonuclease sensitive sites of the hnRNA (between the 30S subparticles) are (partly) covered with a completely distinct and heterogeneous group of proteins. The poly(A)-segment is also partly protected by specific proteins. Other — more or less similar — models of hnRNP structure have been published by Sekeris and Niessing (12), Kinniburgh et al. (42) and Samarina et al. (26, 58).

In addition to a group of proteins involved in protection or stabilization, hnRNP may also contain accessory proteins involved in the processing of hnRNA (25). Niessing and Sekeris (72) described the presence of a nuclease activity in 30S hnRNP particles which *in vivo* might be involved in the cleavage of hnRNA. The same authors also described the presence of two poly(A) polymerase activities in the RNP particles from rat liver nuclei and the occurrence of a ribohomopolymer synthetase which can polymerize ATP, GTP, UTP and CTP to the corresponding ribohomopolymers (94).

In examining the current literature it is apparent that the function of the proteins associated with hnRNA is still unexplained. It is tempting to speculate that they are involved in such poorly understood processes as the conversion of nuclear precursors to functional cytoplasmic mRNA and the transport of processed mRNA sequences into the cytoplasm.

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